EFFECTS OF SOYBEAN PHYTOESTROGENS ON THE DEVELOPMENT OF AVIAN REPRODUCTIVE SYSTEM

ผลของไฟโตเอสโตรเจนในถั่วเหลืองต่อการพัฒนาระบบสืบพันธุ์ในสัตว์ปีก

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Abstract

Many poultry production farms now widely use soybean as a protein source in animal feeds, especially organic farming, as part of a general trend towards greater high-quality plant-based protein. Nevertheless, there have been concerns of such soybean as an animal feed ingredient. A major challenge is that endocrine disruptors (EDs) called phytoestrogens, a term is used to describe a sort of estrogen-like chemical found in plant which can mimic the function of hormone estrogens leading to estrogen-like consequences. Phytoestrogens contained in soybean are classified as the anti-nutritive factors (ANFs) which cannot be detoxified by several treatment methods. Thus, poultry fed soybean can play an important role in anatomical and physiological development of avian reproductive system. This paper offers the current status of the effects of phytoestrogens in soybean on the development of avian reproductive system. The results indicated that the compounds contained in soybean were capable of enhancing growth and structural development when poultry are fed in the proper level. In contrast, improper amount of soybean, which contains several isoflavones particularly genistein and daidzein, was reported their negatively developmental effects.

Keywords: Soybean, Phytoestrogens, Avian, Reproductive system

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บทคัดย่อ

อุตสาหกรรมการผลิตสัตว์ปีกในปัจจุบันมีการใช้ถั่วเหลืองอย่างกว้างขวางในฐานะวัตถุดิบสำหรับอาหารจำพวกโปรตีนในอาหารเลี้ยงสัตว์โดยเฉพาะอย่างยิ่งการเลี้ยงแบบเกษตรอินทรีย์ นับว่าเป็นแนวโน้มอันนำไปสู่การใช้แหล่งโปรตีนจากพืชที่มีคุณภาพสูงมากขึ้น แต่อย่างไรก็ตามการใช้ถั่วเหลืองเป็นอาหารสัตว์นั้นมีโจทย์ท้าทายสำคัญข้อหนึ่งคือสารรบกวนการทำงานของต่อมไร้ท่อ (EDs) ที่พบในถั่วเหลือง เรียกว่า ไฟโตเอสโตรเจน ซึ่งเป็นสารจากพืชที่มีลักษณะโครงสร้างคล้ายกับฮอร์โมนเอสโตรเจน ทำให้กลไกการทำงานและผลลัพธ์ที่ได้นั้นมีความคล้ายคลึงกับการออกฤทธิ์ของฮอร์โมนเอสโตรเจน นอกจากนี้ไฟโตเอสโตรเจนที่พบในถั่วเหลืองจัดว่าเป็นสารรบกวนการทำงานของต่อมไร้ท่อซึ่งไม่สามารถกำจัดโดยกระบวนการแปรรูปต่างๆ ได้ ดังนั้น เมื่อสัตว์ปีกได้รับถั่วเหลืองเป็นอาหาร ไฟโตเอสโตรเจนในถั่วเหลืองจึงมีบทบาทสำคัญต่อกายวิภาคศาสตร์และสรีรวิทยาการเจริญของระบบสืบพันธุ์ในสัตว์ปีก บทความนี้จึงได้นำเสนอผลของไฟโตเอสโตรเจนในถั่วเหลืองต่อการเจริญของระบบสืบพันธุ์ในสัตว์ปีก โดยได้ข้อสรุปว่าไฟโตเอสโตรเจนในถั่วเหลืองสามารถเพิ่มการเจริญเติบโตทางโครงสร้างในระบบสืบพันธุ์ในสัตว์ปีกได้หากได้รับในปริมาณที่เหมาะสม ในทางตรงกันข้าม การได้รับถั่วเหลืองที่มีไฟโตเอสโตรเจนหลายชนิดโดยเฉพาะจีนิสทีนและเดดซีนในปริมาณที่ไม่เหมาะสมจะนำมาซึ่งผลกระทบต่อการเจริญที่ผิดปกติของสัตว์ปีก

คำสำคัญ: ถั่วเหลือง ไฟโตเอสโตรเจน สัตว์ปีก ระบบสืบพันธุ์

Introduction

Endocrine system is important for physiological processes found in animals and plants (Bennink & Boerrigter, 2003). From a baby through adulthood, endocrine glands produce and secrete various hormones along the bloodstream travelling to their distant target tissues and binding to their receptors for signalling cascade induction which regulate or modify many physiological functions including growth, development, metabolism and reproductive activities etc. (Khetan, 2014).

From the renowned publication named "Our Stolen Future" in 1996, the conception of endocrine disruption has first emerged as xenobiotic compounds in animals (Colborn, Dumanoski & Myers, 1996; Khetan, 2014). According to European Commission (2016), endocrine disruptors (EDs) defined as "exogenous substances or mixtures that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations". In fact, the term EDs were initially the well-known issue after the report Rachel Carson's Silent Spring in 1962 which linked between dichlorodiphenyltrichloroethane (DDT) and the environmental effects on wildlife health problems including eggshell thinning, dysmorphism and population decline (Fry, 1995). In addition to DDT, several substances, for example herbicides, plasticizers and plant compounds, had been defined as EDs according to their reproductive effects in various species (Khetan, 2014).

According to Carpenter, Arcaro & Spink (2002) and Vandenberg et al. (2012), endocrine disruptors (EDs) are able to act alongside or additively with other endogenous hormones and exogenous chemicals even though some of the EDs have low-dose effects in the body. In addition, there are some of the EDs, which have specific dose-responses. Unlikely, the typical dose-response curve, which the toxicologists are accustomed to thinking for a long time, demonstrates the increase in response depending on higher dose (Welshons et al., 2003; Khetan, 2014). It is important to be aware of each individual dose in order to determine the toxicity of individual chemicals (Khetan, 2014). Thus, this paper provides an awareness of the effects of phytoestrogens found in soybean on the development of avian reproductive system because soybean meal has widely been known as a protein source in animal feeds for poultry. This is due to the fact that soybean meal is abundant of high-quality plant-based proteins, which are consisted of numerous amino acids. Such amino acids are vital for protein building blocks for poultry (Cromwell, 2016). Soybean meal is a by-product from the soybean oil industry due to the advance of solvent processing technologies in order to produce soybean meal by eliminating the oil (Cromwell, 2016). However, every processing of soybean must be processed appropriately to ensure that all anti-nutritional factors (ANFs) are removed in order to feed poultry. Unfortunately, there is a clear concern with the processing methods that cannot detoxify phytoestrogens contained in soybean meal.

This paper has been examined through a number of studies and the previous experiments, including such key topics as the introduction to soybean phytoestrogens, the functional mechanism of phytoestrogens, and their developmental effects on avian reproductive system in order to attract awareness to the potential effects that soybean phytoestrogens have on development of avian reproductive system.

Soybean Phytoestrogens

Phytoestrogens, which are estrogenic compounds presented in plants such as soybean, are believed as plant defense mechanism in order to protect against insects (Rochester & Millam, 2009). Many phytoestrogens found in plants are divided into three main groups, which include coumestans, lignans and isoflavones (Adlercreutz, 1995). Particularly, isoflavones, which are found in soybean, represent one of the most important studied groups of phytoestrogens, including genistein, daidzein and their glucosides (genistin and daidzin) (Mostrom & Evans, 2012). The ratio of isoflavones presented in soybean products depends on the variety of soybean products; however, such products generally found more genistein than daidzein (Hendrich et al., 1998).

The term “soybean” refers to Asian plant *Glycine max*, which is an economically important grain legume crop (Dei, 2011). Soybean was introduced to Argentina, Brazil, China, India and United States later become known as the top producers, although, it was first cultivated in East Asia (Dei, 2011; Dourado et al., 2011).

The major driving force stimulated the production and consumption of soybean all over the world is the use of soybean meal as high-quality source of protein and unsaturated fatty acids for utilising energy by the animal in livestock feed industry (Blair, 2008; Dei, 2011). Generally, soybean meal provides approximately 150 μg daidzein per gram and 250 μg genistein per gram (Dixon & Ferreira, 2002; Stevenson, 2007). However, phytoestrogens contained in soybean can differ greatly from crop to crop, region to region (Stevenson, 2007). Soybean that used for feed formulation is produced into full-fat soybean, soybean meal and oil. This is due to the fact that raw soybean is unable to feed directly because anti-nutritive factors (ANFs) were numerous found in unprocessed soybeans (Dei, 2011). As previously mentioned, soybean meal has long been considered as one of the most accepted source of high-quality protein and energy in poultry feeds (Cromwell, 2016). Nowadays, apart from soybean meal, the amount of full-fat soybean and feed-grade soybean oil continue to increase gradually because their high energy content and the advanced development of treatments to destroy the levels of ANFs (Dei, 2011; Dourado et al., 2011; Cromwell, 2016). As shown in Table 1, the major ANFs presented in soybean can be eliminated by heat treatment such as phytohaemagglutinins (lectins) and proteases inhibitors (Dei, 2011). Unfortunately, there is a clear limitation with the methods of treatment that cannot detoxify phytoestrogens contained in soybean.

Table 1 Major anti-nutritive factors (ANFs) are found in soybeans

Anti-nutritional factors	Effects	Detoxification process	References
Proteases inhibitors	- Inhibition of the function of digestive enzyme such as trypsin or chymotrypsin - Induction of pancreatic hypertrophy	- Heat treatments - Germination process - Fermentation	Dei, 2011, Dourado et al., 2011
Phytohaemagglutinins (Lectins)	- Erythrocyte agglutination - Diminished absorption of intestinal cells	- Heat treatments	Liener, 1994, Dourado et al., 2011
Phytoestrogens	Reproductive tract enlargement	-	Dei, 2011

Source: adapted from Dei (2011: 22)

Functional mechanism of phytoestrogens

Phytoestrogens can competitively bind estrogen receptors (ERs) situated in nuclear membrane with endogenous estrogens but generally less potential than endogenous estrogens. (Belcher & Zsamovszky, 2001; Ratna, 2002). In terms of functional mechanism of phytoestrogens, both endogenous estrogens and phytoestrogens consisted of the phenolic ring enable them to bind directly particular receptors or cell surfaces forming "ligand-receptor complex" in cytoplasm, transport to nucleus in order to manipulate the control regions for transcription of the DNA or small RNA (sRNA) and finally activate the expression of the specific genes (Korach et al., 1997; Sirotkin & Harrath, 2014) (Figure 1)

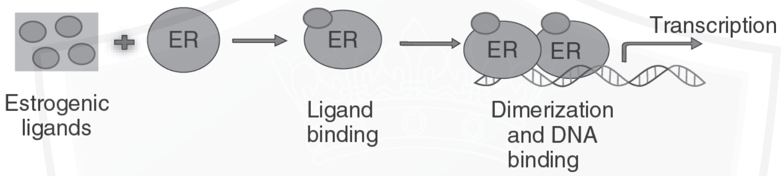

Figure 1 The general mechanism of phytoestrogens

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In most female *vertebrate* animals, estradiol (17-β-estradiol) is one of the most potent and main forms of estrogens found in the active reproductive period, which is usually released by the ovaries (Bennink & Boerrigater, 2003; Stevenson, 2007). Estradiol would be active in very low concentration in bloodstream which is generally between 0.00001 and 0.0009 ppm (Vandenberg et al., 2012). As previously described, phytoestrogens are able to bind estrogen receptors according to structural similarity with estradiol and induce a response that mimics the action of endogenous hormones leading to estrogen-like consequences (Stevenson, 2007) Thus, every process is controlled and maintained by estrogens (estradiol) can be influenced by phytoestrogens (Wang, 2002). This is due to the fact that numerous estrogen receptors found in many different tissues and organ systems, particularly, reproductive organs and accessory sexual glands. (Sirotkin & Harrath, 2014). However, the effects of phytoestrogens mentioned above rely on many factors that need to be considered, for instance, endogenous hormones levels, phytoestrogens levels, types of tissues, types of estrogen receptors, etc. (Gehm et al., 2004; Rochester & Millam, 2009).

The effects of soybean phytoestrogens on the development of avian reproductive system.

During embryonic period, sex determination and differentiation of avian reproductive structures require sex hormones (estrogens) driving the proper developmental consequences for each structure which prominently developed in the left side of the body (Intarapat & Stern, 2013; Intarapat, Sailasuta & Satayalai, 2014). According to Ottinger et al. (2005) and Stevenson (2007), the effects of embryonic exposure to estrogen-like compounds were divided into short-term and long-term effects. The obvious effects for instance hatching period and neuroendocrine are classified in short-term effects. In the case of long-term effects, some estrogen-like compounds might bring about oviduct abnormalities in the adult animal. For example, the retention of Müllerian ducts which normally regress on right side in female and both sides in male, demasculinized male left gonad which maybe form ovary-like tissue in testis called ovotestis, reduced egg production, drastically altered carbonic anhydrase distribution in the shell gland, and production of shell-less eggs (Holm et al., 2001; Brunstrom, Axelsson & Halldin, 2003; Stevenson, 2007; Intarapat, Sailasuta & Satayalai, 2014).

According to Intarapat, Sailasuta & Satayalai (2014) and Zhengkang et al. (2006), isoflavones (genistein and daidzein) are prominent EDs treated in avian species to study their reproductive and developmental effects which has been shown on different sorts of avian species (Table 2)

Table 2 Reproductive and developmental effects of isoflavones (phytoestrogens) on different avian species

	Avian models	Age (days/months)	Dose	Effects	References
Genistein	Quails	Japanese quail *(Coturnix japonica)* eggs	16, 24 μg/g, in ovo injection	- Cause a retention of Müllerian ducts in both sexes - Cause a demasculinization in male left gonad	Intarapat, Sailasuta & Satayalai, 2014
	Chickens	*In vitro* experiment with roosters (*Gallus gallus domesticus*) Leydig cells	5-50 μM, Leydig cells were incubated with genistein.	- Cause a suppression of basal and LH-stimulated testosterone secretion by Leydig cells	Opalka et al., 2004
	Ganders	*In vitro* experiment with Bilgoraj gander Leydig cells	5μM, 50μM, Leydig cells were incubated with genistein.	- Cause a suppression of secreting testosterone by incubated Leydig cells	Opalka et al., 2006, 2008, 2012
			Leydig cells were incubated with 50μM genistein, then incubated with 20 μM testosterone (T) precursors	- Cause a suppression of the stimulatory effects of testosterone precursors	
		1 day-old, males (Bilgoraj ganders: *Anser domesticus*)	383.55 mg/g in feed, during growth period 118.54 -151.19 mg/g in feed, before and during mating period	- Cause a reduction of ejaculate volume and percentage of normal sperm morphology in Bilgoraj ganders - Cause a height of the seminiferous epothelium in Bilgoraj ganders	

Table 2 Reproductive and developmental effects of isoflavones (phytoestrogens) on different avian species (cont.)

	Avian models	Age (days/months)	Dose	Effects	References
Daidzein	Quails	35 day-old, females 7 months old, females	3 mg/kg in feed	- Stimulate an increase egg-laying rate and blood triiodothyronine (T3) levels	Ke, Wang & Han, 2002, Zhengkang et al., 2006
		7 months old, females	6 mg/kg in feed	- Stimulate an decrease egg-laying rate	
		12 months old, females	6 mg/kg in feed	- Stimulate an increase egg-laying rate	
	Chickens	330 day-old, females	3 mg/kg in feed	- Stimulate an increase of blood triiodothyronine (T3) and progesterone levels in laying hens	Wistedt et al., 2012, Yin et al., 2004, Zhengkang et al., 2006
		N/A	10, 20, and 40 mg/kg in feed	- Compositional change of egg in layers (egg cholesterol content and egg yolk cholesterol concentration were reduced)	
		242 and 330 day-old, females	3 mg/kg in feed	- Stimulate an increase egg-laying rate in laying hens	
		60-72 week-old, females (Lohmann Selected Leghorn: LSL) and Lohmann Brown: LB)	50 mg/kg in feed	- Stimulate an increase of shell gland carbonic anhydrase activity in laying hens	
	Ganders	In vitro experiment with Bilgoraj gander Leydig cells	5μM, 50μM, Leydig cells were incubated with genistein.	- Cause a suppression of secreting testosterone by Leydig cells	Opalka et al., 2006, 2012
	Shaoxing ducks (Anas platyrhyn-chos)	415-day-old, females	5 mg/kg in feed for 9 weeks	- Compositional change of egg (Egg mass increased, yolk or albumin ratio reduced)	Zhao et al., 2004

According to several experiments summarised in Table 2, genistein and daidzein containing in animal feed, *in vitro* experiment and *in ovo* injection in avian species showed the interference of reproductive functions and developmental processes. Therefore, soybean which contains genistein and daidzein might be able to affect the reproductive malfunction and malformed development in poultry. However, these effects depended on *species,* specific *age and developmental times*.

Brunstrom, Axelsson & Halldin (2003) classified the avian species into two major *species* which are affected by sex hormones during developmental times: precocial and altricial avian species. Precocial avian species, such as the domestic chickens and ducks, were affected by sex hormones particularly during embryonic period development. In contrast, the zebra finch was one of the altricial species which were affected both in the embryonic period development and the period after hatching. Thus, it is possible that whether an animal feed fed to females during the egg-laying period is found to contain phytoestrogens, altricial species were more likely to be affected by them. According to Intarapat, Sailasuta & Satayalai (2014), phytoestogens are maternally transferred to the egg through the circulatory system. Vitellogenin is synthesised to act as egg yolk precursor in the bloodstream of egg-laying avian species (Saitoh et al., 2004). Both dietary isoflavones and vitellogenin are cotransported as complexes into the oocyte which most of yolk components are generally transferred into the oocytes from bloodstream before ovulation (Saitoh et al., 2004). Therefore, there have been concerns of such soybean as an animal feed. The main challenge is that the effects of exposing phytoestrogens on growth and reproductive functions of the offspring are needed to be taken into consideration in a long-term (Daston et al., 1997).

Conclusions

As soybean is well known as one of the feed ingredients for poultry, some compounds contained in soybean are normally capable to enhance the growth and structural development when they are fed in the proper level. On the other hand, improper amount of soybean, which contains several isoflavones especially genistein and daidzein, is reported their negatively reproductive and developmental effects. Basically, isoflavones are structurally similar to estrogen, which regulates the normal female reproductive development, and consequently induce the estrogen-like developmental activities. Excess estrogen-like compounds, known as EDs, affect the remaining activation of the estrogen-dependent processes leading to malformation or failure in developmental mechanisms. Moreover, the EDs is the exception of the normal toxicological plots which is dose-dependent toxicity because their toxicity can be varying as mentioned above. Therefore, the use of soybean as animal feed should be considered the appropriate concentration and also other system development in the future.

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